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Soft tissue volume augmentation at dental implant sites using a volume stable three-dimensional collagen matrix – histologic outcomes of a preclinical study.

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Abstract

Aim: To test whether or not soft tissue augmentation with a collagen matrix (VCMX) leads to a similar increase in ridge width around dental implants compared to the use of an autogenous subepithelial connective tissue graft (SCTG).

Materials and methods: In 12 dogs, immediate dental implants were placed with simultaneous guided bone regeneration. Three months later, soft tissue volume augmentation was performed by randomly allocating three treatment modalities to these sites (VCMX, SCTG, sham-operated group (control)). Dogs were sacrificed at 1 (n=4), 2 (n=4) or 6 months (n=4). Descriptive histology and histomorphometric measurements for soft tissue thickness were performed on non-decalcified sections.

Results: The horizontal soft tissue thickness was maximal at the most coronal level (alveolar crest) at 1 month (VCMX: 2.1mm±1.6mm; SCTG: 2.5mm±1.7mm; p=0.877) and decreased until 6 months. At 6 months, the greatest mucosal thickness was at a level 3.5mm below the crest (VCMX: 0.8mm±0.3mm; SCTG: 0.7mm±0.2mm) (p=0.754). Control sites revealed no relevant soft tissue augmentation at any level and any time-point. Tissue integration for VCMX and SCTG were favorable with minimal inflammatory reactions.

Conclusions: Soft tissue volume augmentation at implant sites was obtained to a similar extent using VCMX and SCTG up to 2 months. Thereafter, degradation and remodeling processes were enhanced leading to a minimal increase of soft tissue thickness at 6 months for VCMX and SCTG.

Clinical relevance:

Scientific rationale for the study: Soft tissue volume augmentation around dental implant is usually performed using the patient's own tissue. This therapy is associated with an increased morbidity due to the second surgical site. Data for soft tissue substitutes serving as replacements for connective tissue grafts (SCTG) are scarce.

Principal findings: The volume stable cross-linked collagen matrix rendered an increase in ridge width similar to SCTG at dental implant sites.

Practical implications: Based on previously published volumetric and histologic outcomes and the present data, this collagen matrix may be used as an alternative for autogenous soft tissue in the future.

Introduction

Soft tissue grafting procedures are routinely performed for a variety of clinical indications to increase the soft tissue volume in pontic sites and around dental implants, mainly for esthetic purposes (Studer et al., 2000, Kinsel and Capoferri, 2008, Schneider et al., 2011, De Bruyckere et al., 2015). According to a clinical study in humans, soft tissue volume grafting is responsible for 43% of the final volume and therefore serves as a major contributor to an optimal final outcome in single-tooth implant sites (Schneider et al., 2011). Based on scientific evidence autogenous grafts are considered to be the gold standard for soft tissue volume augmentation rendering an increase ranging between 1.7 and 3.2mm over time (Thoma et al., 2014, Thoma et al., 2009). Major disadvantages and limitations associated with the use of autogenous grafts, are the harvesting procedure and variations in quality and quantity of tissue that is available for grafting. In order to overcome these drawbacks and to standardize grafting procedures, soft tissue substitutes were introduced in the past. These devices were predominantly used to increase the width of keratinized tissue, for ridge preservation and recession coverage (Jepsen et al., 2013, Jung et al., 2013, Wei et al., 2000, Schmitt et al., 2013). In terms of a more extensive volume increase, these devices cannot be recommended rendering a limited soft tissue volume gain only (Simion et al., 2012, Jung et al., 2011). Therefore, a volume stable cross-linked collagen matrix (VCMX) was developed recently and evaluated in a number of in vitro and preclinical studies (Thoma et al., 2015, Mathes et al., 2010, Thoma et al., 2011a, Thoma et al., 2010, Thoma et al., 2012). The VCMX demonstrated favorable mechanical and biological properties, enhancing connective tissue formation within the collagen matrix body, undergoing simultaneous remodeling process and partial degradation (Thoma et al., 2015). In terms of soft tissue volume increase, the VCMX provided a similar gain in ridge width based on histologic and volumetric outcome measures when applied to chronic ridge defects (Thoma et al., 2011a, Thoma et al., 2010). Clinically, the number of patients treated with dental implants increases and due to esthetic needs, soft tissue volume augmentation might be a desired therapy. Currently, no data are available for the use of soft tissue substitutes to increase soft

tissue volume at implant sites and histologic data for VCMX placed on the surface of implants and regenerated peri-implant bone are missing.

The aim of the present study was, therefore, to test whether soft tissue augmentation with a volume stable cross-linked collagen matrix leads to an increase in ridge width at dental implant sites similar to those obtained by an autogenous subepithelial connective tissue graft.

Material and Methods

Study Design

The present study was designed as a randomized controlled experimental study and conducted in accordance with the OECD Good Laboratory Practice regulations, ENV/MC/CHEM (98) 17, with the European Good Laboratory Practice regulations, 2004/10/EC Directive and with the United States Food and Drug Administration Good Laboratory Practice regulations, 21 CFR 58. The protocol was approved by the local ethical committee of NAMSA (Lyon, France) on September 9, 2013. Twelve adult male beagle dogs (about 2 years old), weighing 11 ± 3 kg, were kept in a purpose-designed room for experimental animals and fed a soft diet during the entire study period.

Animal preparation and medication

The dogs were fasted for food 12 to 24 hours before the surgeries. Spiramycin and metronidazole (Buccoval®, Sogeval) were administered per os and penicilline, procaine and benzathine (Duplocilline®, Intervet S.A) were injected subcutaneously the day before surgery. After an intramuscular injection of medetomidine (Dorbene Vet®, Pfizer), anesthesia was performed by intravenous administration of ketamine (Ketamine 1000®, Virbac) and atropine (Atropinum Sulfuricum, Aguettant) followed by inhalation of an O2 – isoflurane mixture (Isoflo®, Axience). A pre-operative subcutaneous injection of carprofene (Rimadyl®, Pfizer) and buprenorphine (Buprecare®, Axience SAS) was additionally administered. An intravenous infusion with an electrolyte solution was performed during surgery (Ringer lactate, CEVA Santé Animal). The dogs were placed on a heating pad and the mandibles disinfected by application of a 0.2% chlorexhidine solution (Cooper). Prior to the beginning of the surgeries, injections of 2% lidocaïne with adrenaline (Lidocaïne adrenaline®, Aguettant) were administered for each hemi-mandible. An overview on the schedule of the study is displayed in Fig. 1.

Extractions and implant placement

Following elevation of a muco-periosteal flap on the lingual and buccal side between M2 and P1, all mandibular mesial roots of P3, P4, and M1 were extracted and

the buccal bone plates removed (Fig. 2A). The distal roots of each P3, P4, and M1 were root canal treated according to a previously described procedure (Thoma et al., 2010). A two-piece dental implant (Straumann BoneLevel, 3.3mmx8mm, Institut Straumann AG, Basel, Switzerland) was placed in the area of the mesial root of P3, P4 and M1 with the implant shoulder at the level of the lingual bone crest (Fig. 2B). The buccal peri-implant dehiscence and infrabony defects were regenerated with demineralized bovine bone mineral (Bio-Oss®, (0.25-1mm) Geistlich Pharma AG, Wolhusen, Switzerland) (Fig. 2C). A native collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was applied to cover the augmented area (Fig. 2D). Following rinsing with sterile saline and releasing incisions within the periosteum, primary wound closure was obtained with non-resorbable sutures (GoreTex 5-0®, GoreTex, Germany) (Fig. 2E).

Soft tissue augmentation

Soft tissue augmentation surgeries were performed on one side of the mandible at 25 weeks and repeated on the contralateral side at 29, 42 or 45 weeks (both post implant placement) (Fig 3a). For that purpose, sulcular incisions were made around P1 and P2, the remaining roots of P3, P4 and M1 and partial-thickness flaps elevated, thereby preparing a pouch on the buccal side of the implants. Subsequently, three treatment modalities were randomly applied to the defect sites according to a computer-generated randomization list:

- Volume stable cross-linked collagen matrix made of porcine collagen (VCMX) (Fibro-Gide, Geistlich Pharma AG, Wolhusen, Switzerland)
- autogenous subepithelial connective tissue graft (SCTG)
- sham-operated site (control)

The VCMX is designed as a matrix for submerged healing and to serve as a replacement for the autogenous subepithelial connective tissue graft (Thoma et al., 2016). The matrix is characterized by a loose network, is cross-linked and made of porcine collagen. It combines the stability of cross-linked collagen membranes (Brunel et al., 1996) and the favorable tissue integration of non-cross-linked collagen membranes (Thoma et al.,

2011b), but does not have a membrane function. The VCMX (dimension: 10 mm x 7.5mm x 5mm) was positioned in the pouch underneath the elevated buccal flap. The VCMX was stabilized with a horizontal mattress suture connecting it to the lingual flap (Dafilon® 5-0, B. Braun Melsungen AG, Melsungen, Germany)(Fig. 3b1). The SCTG was harvested from the lateral part of the palatal vault (Thoma et al., 2010). Fatty, and glandular tissue, as well as remnants of the epithelium were removed, resulting in a dimension (thickness) similar to the VCMX. Compression with a sterile gauze and 3 to 4 single sutures (Dafilon® 5-0, B. Braun Melsungen AG, Melsungen, Germany) were used to reposition the epithelial coverage at the palatal donor site. The SCTG was then positioned in the buccal pouch under the elevated flap and immobilized using a horizontal mattress suture connecting it to the lingual flap (Dafilon® 5-0, B. Braun Melsungen AG, Melsungen, Germany) (Fig 3b2). In control sites no further augmentation was performed (Fig 3b3). Subsequently, in all sites, periosteal releasing incisions were made to allow for a tension-free primary wound closure using one horizontal mattress suture and four to five single sutures (Dafilon® 5-0, B. Braun Melsungen AG, Melsungen, Germany) (Fig. 3c). Suture removal was performed 14 days later and the sites were macroscopically inspected.

Sacrifice

At 1, 2 and 6 months post soft tissue augmentation, euthanasia was performed by a lethal injection of a barbiturate (Dolethal®, Vetoquinol). The hemi-mandibles were sampled en bloc and fixed in 4% neutral buffered formalin for histopathologic analysis.

Histologic preparation

After initial fixation, the hemi-mandibles were dissected into individual blocks with a band saw (one block per site) and fixed again in 4% neutral buffered formalin. After complete fixation, the sites were dehydrated in alcohol solutions of increasing concentration, cleared in xylene and embedded in polymethylmetacrylate (PMMA). One central bucco-lingual cross section through the dental implant was performed by a

microcutting and grinding technique (approximately 40 to 50µm thick) (Donath and Breuner, 1982). The histologic slides were stained with a modified polychromatic stain (Paragon) for qualitative, semi-quantitative and quantitative histopathologic analysis.

Descriptive histology

The histologic sections were analyzed by light microscopy (Nikon Eclipse 80i, objectives: x2, x10, x20, x40 and x60) equipped with a color image analyzing system Tribvn® (Tribvn, Chatillon). A qualitative and semi-quantitative evaluation of the tissue effects was performed in adaptation to ISO 10993 6 and in compliance with the standard nomenclature of the International Society for Stereology (Exner, 1987).

Histomorphometric measurements

The histomorphometric outcomes were assessed according to Fig. 4. For that purpose, the borders of the prepared pouch underneath the buccal flap augmented with the VCMX, the SCTG or left empty (control) were defined. A horizontal line (bucco-lingual axis) was drawn at the top of the lingual crest, perpendicularly to the dental implant (green line in Fig. 4). A vertical line (corono-apical axis) was then drawn perpendicularly to this horizontal line, at the level of the top of the threads of the implant (orange line in Fig. 4). Four additional lines (blue) were drawn perpendicularly to the vertical line at four different levels (1.5mm, 3.5mm, 5.5mm and 7.5mm) below the horizontal line. The ridge width (bucco-lingual axis) was measured along these horizontal lines (green and blue lines) for implant, bone marrow, bone substitute material, bone, pouch with the VCMX, SCTG or empty control (=augmented soft tissue thickness) and native soft tissue (Fig. 4).

Statistical analysis

Data was summarized in terms of means and standard deviations. The local tissue effects and performance were compared among groups and time-points as follows: VCMX versus SCTG and control; SCTG versus control; for each group, comparison between the different time periods (1, 2 and 6 months). The local tissue effects were evaluated based on comparison of the semi-quantitative histopathologic inflammatory parameters. No

statistical analysis was performed for these parameters. The performance evaluation was based on the semi-quantitative histopathologic evaluation and the quantitative histomorphometric evaluation. For that purpose, a statistical analysis was conducted (5% risk) with a statistical software (Software SPSS version 19.0, SPSS inc.) for the augmented soft tissue thickness.

Results

Clinical and macroscopic findings

The dogs remained healthy and neither systemic complications nor local intolerances at the augmented sites occurred during the entire study period. Some soft tissue dehiscences or delayed wound healing, however, occurred after extractions and implant placement (one side in two dogs) as well as after soft tissue augmentation surgeries (one side in two dogs). These observations were not correlated to any treatment group and all sites healed without further treatment.

Descriptive histology

In general, inconsistent signs of osseointegration of the bone graft particles were observed. Frequent signs of fibrous encapsulation and/or loss of the bone graft particles were noted. A moderate to marked grade horizontal and/or vertical bone regeneration was observed up to 6 months irrespective of the groups. This often led to buccal implant surfaces being covered by soft tissues. In some sites the native collagen membrane persisted over time and was still visible up to 6 months.

VCMX: At 1 month, a slight to moderate infiltration of macrophages and lymphocytes and a limited number of plasma cells was observed (Fig 5a). The VCMX was integrated and slightly to moderately degraded (Fig. 5b). A slight to moderate grade of soft tissue augmentation and vertical bone growth was observed. At 2 months, the VCMX network was integrated, moderately degraded and slightly infiltrated by macrophages, lymphocytes and plasma cells. A slight soft tissue augmentation and a moderate grade of vertical bone growth were observed. At 6 months, no signs of local inflammation were visible anymore. A null to slight soft tissue augmentation and a moderate to marked grade of vertical bone growth were evident. In addition, discrete signs of mineralization were noted in one out of eight sites. The VCMX was completely integrated and markedly to severely degraded (Fig 5c).

SCTG: At 1 month, no significant local inflammation was observed (Fig. 5d). The SCTG was integrated, remodeled and degraded to a moderate to marked degree. A slight

soft tissue augmentation and a marked grade of vertical bone growth occurred. One site demonstrated discrete signs of mineralization. From 2 months on, no local inflammation or mineralization was observed (Fig 5e). The SCTG was not clearly identifiable and was considered fully degraded at 2 months. No obvious signs of soft tissue augmentation were evident, whereas a marked grade of bone growth had occurred. At 6 months, no soft tissue augmentation, but a moderate to marked grade of bone growth was evident (Fig 5f).

Control: The tissues were healed at 1 month. Soft tissue augmentation, local inflammation and signs of mineralization were not observed at any time-point. Bone formation, however, increased over time up to a marked grade at 6 months (Fig 5g).

Histomorphometric outcomes (horizontal soft tissue thickness)

All data are displayed in Table 1 and Appendix 1. Other than reported, no statistically significant differences were observed between the groups for any of the outcomes measures (Table 2; $p>0.05$).

In general, the augmented soft tissue thickness for VCMX and SCTG were highest at the level 0mm at 1 month and decreased to the level 7.5mm up to 6 months. Control sites revealed no relevant augmented soft tissue thickness at any level and any time-point (range: 0.07mm to 0.66mm).

At 1 month, VCMX and SCTG showed overall higher values of augmented soft tissue thickness at all apico-coronal ridge height levels compared to the control group. At 1.5mm and 3.5mm, the VCMX group had significantly more soft tissue gain compared to the control group ($p= 0.011$; $p= 0.019$), whereas the SCTG group had significantly more soft tissue gain at 0mm and 1.5mm compared to the control group ($p= 0.019$; $p= 0.012$) (Table 2).

At 2 months, the VCMX and SCTG groups showed overall higher values of augmented soft tissue thickness at all apico-coronal ridge height levels compared to the control group, even though VCMX and SCTG values decreased when compared to 1 month. The differences between VCMX and control were significant at 3.5mm ($p=0.030$) (Table 2).

At 6 months, VCMX and SCTG demonstrated a higher gain of augmented soft tissue thickness at all apico-coronal ridge height levels compared to the control group. Again, the overall number of VCMX and SCTG had decreased from 2 months. At 3.5mm, the VCMX group yielded significantly higher values of augmented soft tissue thickness compared to the control group ($p= 0.015$). At 5.5mm, the SCTG group yielded significantly higher values compared to control group ($p= 0.025$) (Table 2.)

Other than reported, none of the differences for augmented soft tissue thickness were statistically significantly different between the groups ($p>0.05$) (Table 2).

Discussion

The present experimental study revealed that i) soft tissue volume increase could be obtained using VCMX and SCTG, ii) control sites did not show any gain in soft tissue thickness, iii) augmented soft tissues underwent major remodeling processes over time and, iv) these remodeling and degradation processes lead to a decrease in the augmented soft tissue thickness between 2 and 6 months for VCMX and SCTG.

The stability and thickness of augmented soft tissues is considered to be a contributing factor for the success of dental implants and helps to facilitate the choice of the reconstructions (Brito et al., 2014, Jung et al., 2008). This is based on clinical data that demonstrate soft tissue surgeries contribute to more than 40% of the final horizontal/buccal volume with minimal changes up to one year (Schneider et al., 2011), as well as the critical threshold value of the mucosal thickness being 2mm (Jung et al., 2007). In case less than 2mm of buccal mucosal thickness is present, the use of a metal abutment may lead to a greyish discoloration of the mucosa, whereas all-ceramic reconstructions are more favorable in terms of esthetics (Jung et al., 2008, van Brakel et al., 2011). This in turn means that a certain mucosal thickness may be desired to leave more freedom in the choice of the reconstruction material. Autogenous SCTGs are still considered to be the gold standard and data on soft tissue substitutes for soft tissue volume augmentation are limited to preclinical data or limited to devices not primarily intended to be used for volume increase (Thoma et al., 2014). The outcomes of the present study revealed a similar and successful increase in mucosal thickness at implant sites at 1 and 2 months. This is in line with previous data comparing the same two treatment modalities (VCMX and SCTG) for soft tissue volume augmentation in chronic ridge defects (Thoma et al., 2011a). In that study, a volume increase at 1 month and stability of the augmented region up to 3 months was documented. That study also revealed that both soft tissue grafts underwent remodeling and degradation processes. For the VCMX, more bone formation and a slightly higher loss of ridge width (in terms of soft tissue) was observed. A different study design was chosen for the present experimental trial. Immediate implant placement with simultaneous GBR was performed.

Even though a healing time of 25 and 45 weeks was initiated prior to soft tissue augmentation - and in contrast to previous data with chronic ridge defects - a more acute situation was present. These sites probably underwent changes in terms of maturation and regeneration of the hard tissue augmentation, which might have contributed to a more reactive environment for the soft tissue grafts on top. In addition, as identified on the histologic slides, the GBR procedure was not as successful as expected. A major part of the applied biomaterials was lost (probably due to encapsulation within the soft tissues) or displaced more apically. This resulted in implant surfaces being exposed to the soft tissues. As clearly visible on the histologic slides, VCMX and SCTG were placed in part on these exposed implant surfaces without underlying bony support (Fig. 5). These two reasons could explain, why both soft tissue grafts, VCMX and SCTG, underwent more extensive remodeling and degradation processes and demonstrated only a limited increase in augmented soft tissue thickness at 6 months. Moreover, in the previously published study (Thoma et al., 2010), the augmented soft tissues (both VCMX and SCTG) had a higher initial thickness (augmented thickness: 10mm, uncompressed). This is in contrast to the present study, where SCTG and VCMX were not folded and had a thickness of roughly 50% (5mm) compared to the previous experimental trial. Still, remnants of the transplanted SCTG and of the matrix body (VCMX) could be identified at the last sacrifice time-point (6 months). This is, for VCMX, in line with a previous study in rats (Thoma et al., 2015). In that study, VCMX was placed in subcutaneous pouches and followed up to 6 months. Similar to the present study, remnants of the matrix could be observed, but the thickness of the VCMX had been decreased. Another explanation for the minimal soft tissue thickness, slightly higher than control sites, could be that the ridge was augmented outside the bony envelope. It has been speculated that in cases where bone regeneration is attempted by overcontouring the ridge (bone regeneration outside the bony envelope), the obtained final ridge width is suboptimal with an increased rate of buccal bone loss or an only limited gain (Garaicoa et al., 2015, Park et al., 2009). In terms of soft tissues, one might speculate that the minimal increase, observed at 6 months, might be due to the

augmentation outside of the bony envelope . SCTGs had been used in the past to augment chronic ridge defects and controlled up to 5 years post insertion of the final fixed dental prosthesis (Sanz Martin et al., 2016). The study revealed stable soft tissues and a minimal decrease in height and volume over 5 years. Control sites without soft tissue grafting demonstrated the same stability as SCTG sites. This long-term stability might reflect that once augmented soft tissues are stimulated by the pontic of a fixed dental prosthesis, these sites might undergo less volumetric changes.

The descriptive histology and semi-quantitative analyses demonstrated that the augmented SCTG integrated and subsequently remodeled and degraded to a marked grade without a significant inflammation at 1 month. At the same time, bone formation had taken place underneath the SCTG. Data of a previous study with a similar set-up (Thoma et al., 2011a), but chronic ridge defects that were augmented, revealed a similar limited inflammatory reaction. The borders to the underlying bone, however, were distinct. This might be explained by the chronic defects, whereas in the present study, remodeling and maturation processes from the underlying GBR materials were ongoing and resulted in a less distinct border towards the bone. From 2 months on in the present study, the SCTG was not clearly identifiable and degraded to a marked grade leaving almost no augmented soft tissue left at 6 months. The native soft tissue, however, increased during the entire study period in all groups. This observation could have in part compensated the loss of the augmented area and might be explained by difficulties to assed the border between augmented and native soft tissue. The observation that soft tissues demonstrate spontaneous thickening and can compensate for missing hard tissue on the buccal side of dental implants has been reported in clinical studies (Benic et al., 2012, Kuchler et al., 2016, Chappuis et al., 2015). In terms of collagen-based soft tissue substitutes and membranes, a variety of preclinical and clinical studies have been published in the past. Based on these experiments, degradation, tissue integration and angiogenesis were predominantly influenced by the composition of the membranes/matrices with more favorable biological attributes for native non-cross-linked collagen-based materials compared to controls (Rothamel et al., 2012, Schwarz et

al., 2006, Rothamel et al., 2004). For VCMX, previous data revealed ongoing long-term remodeling processes up to 6 months in a mouse model (Thoma et al., 2015) and up to 3 months in a canine model (Thoma et al., 2011a). At 1 month, in both studies, the VCMX was fully integrated without clear distinction to the surrounding tissues and thereby demonstrated favorable biologic properties enhancing connective tissue formation. Similar to previous data, a marked degradation was observed up to the 6-month time-point. The VCMX were partially replaced by newly formed connective tissue, but left only a minimal soft tissue thickness. In contrast to SCTG sites, VCMX sites demonstrated a slight to moderate infiltration of inflammatory cells at 1 and 2 months, but not at 6 months. Tissue integration of (non)cross-linked collagen membranes and matrices has been investigated previously. In an ectopic mouse model, two prototype VCMX were implanted in subcutaneous pouches. The inflammatory reaction at 3 and 6 weeks was limited (Thoma et al., 2012). In contrast, similar to the present study, a significantly elevated number of inflammatory reactions in the adjacent soft tissues was observed in a clinical study using a cross-linked membrane (Becker et al., 2009). Whereas in the latter clinical study, this adverse tissue reaction resulted in an early exposition of GBR membranes, the VCMX in the present study did not demonstrate any such outcomes and further healing was uneventful without an elevated number of inflammatory cells at 6 months. In a recent clinical study, biopsies of VCMX were obtained 3 months post soft tissue augmentation. Histologic analyses demonstrated the presence of a limited number of inflammatory cells, but no adverse tissue reactions or an increased rate of dehiscences compared to sites treated with SCTGs (Thoma et al., 2016).

In the present study, histologic slides were analyzed for mineralization within the augmented soft tissues, thereby assessing the biocompatibility and local tolerance of the soft tissue substitute (VCMX) and the transplanted autogenous grafts (SCTG). For both VCMX and SCTG, mineralization was observed in one site only at one time-point only. This underlined that the grafting materials could be safely used without hampering tissue integration and elicited biocompatibility.

From a clinical point of view, the present study indicated that both VCMX and SCTG, could be used for soft tissue augmentation at implant sites. Clinicians need to be aware of ongoing hard and soft tissue remodeling processes that could change the tissue architecture. It remains unknown, however, how the augmented soft tissues would behave if pressure or stimulation is applied by reconstructions once dental implants are loaded or FDPs inserted in these areas. Future research is needed to determine an optimal time-point to start conditioning of augmented soft tissues (with VCMX and SCTG) at implant sites.

Conclusion

The cross-linked volume-stable collagen matrix and the subepithelial connective tissue graft rendered a similar gain in ridge width at sites where implants with simultaneous guided bone regeneration had been placed. The ridge width was predominantly increased at 1 and 2 months for VCMX and SCTG sites. The augmented areas underwent continuous and enhanced remodeling processes, both, on the hard and soft tissue level. At 6 months, most of the VCMX network and the augmented SCTG had been degraded and/or replaced leaving only a minimal increase in soft tissue thickness. VCMX sites demonstrated a slight to moderate infiltration with inflammatory cells during the first 2 months, whereas in SCTG sites, no inflammation was present. Adverse tissue reactions such as local intolerance or issues with biocompatibility were negligible for VCMX and SCTG. Both VCMX and SCTG can be used for soft tissue augmentation at dental implant sites, however, rendering short-term gain in ridge width only.

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Figure legends

Figure 1.
Overview of the study schedule.

Figure 2.
Clinical situation at the time of extraction and immediate implant placement.
A Occlusal view of a lower right mandible with extracted mesial roots of teeth P3, P4, M1.
B Immediate implant placement in the extraction sites.
C Guided bone regeneration performed on the buccal aspect of the implants using deproteinized bovine bone material (DBBM).
D Implant sites and DBBM material covered with resorbable collagen membranes.
E Wound closure using non-resorbable sutures.

Figure 3.
Clinical situation at the time of soft tissue augmentation.
a Occlusal view before the surgical intervention.
b Augmentation sites according to randomization for the respective groups:

1. volume-stable collagen matrix (VCMX) at the central site
2. autogenous subepithelial connective tissue graft (SCTG) at the mesial site
3. sham-operated site (control) at the center of the picture

c Occlusal view after suturing.

Figure 4.
Template used for the histomorphometric measurements depicting the different evaluated areas (bone, bone substitute, non-mineralized tissue, augmented area, covering flap) and the five levels used for the horizontal measurements.

Figure 5.
Histologic slides stained with Paragon of the three groups at the different time points.
a Group VCMX at 4weeks (0.5x magnification).
b high resolution image of VCMX at the center of the augmented area at 4weeks (100x magnification)
c Group VCMX at 24weeks
d high resolution image of SCTG at the center of the augmented area at 4weeks (100x magnification)
e Group SCTG at 8weeks (0.5x magnification).
f Group SCTG at 24weeks (0.5x magnification).

G Group Control (sham-operated site) at 8weeks (0.5x magnification).
AC=adipocytes; BV=blood vessel; CF=covering flap; CT=connective tissue; SCTG=autogenous subepithelial connective tissue graft; VCMX=volume-stable collagen matrix

Table 1.
Summary of the histomorphometric measurements in horizontal thickness of the augmented soft tissue thickness at the different time points (4, 8, and 24 weeks).
Measures are depicted for the three groups SCTG (subepithelial connective tissue graft); VCMX (volume stable cross-linked collagen matrix) and sham-operated site (control) at five different levels perpendicular to the implant axes (0mm, 1.5mm, 3.5mm, 5.5mm, 7.5mm)
Mean, standard deviations (SD), and Medians were calculated with a confidence interval (CI) of 95%.

Table 2.
Inter-group comparison of the augmented areas for the groups SCTG (subepithelial connective tissue graft), control (sham-operation) and VCMX (volume stable cross-linked collagen matrix) were calculated at 4, 8, and 24 weeks at the five different levels.
*Significant p-values.

Appendix 1.

Summary of the histomorphometric measurements in horizontal thickness of the different areas (implant, bone marrow, bone substitute material, bone, native soft tissue) at the different time points (4, 8, and 24 weeks).
Measures are depicted for the three groups SCTG (subepithelial connective tissue graft); VCMX (volume stable cross-linked collagen matrix) and sham-operated site (control) at five different levels perpendicular to the implant axes (0mm, 1.5mm, 3.5mm, 5.5mm, 7.5mm)
Mean, standard deviations (SD), and Medians are calculated with a confidence interval (CI) of 95%.

Table 1

		4 weeks			8 weeks			24 weeks		
		SCTG	VCMX	Control	SCTG	VCMX	Control	SCTG	VCMX	Control
		Mean SD Median 95% CI (low and up)	Mean SD Median 95% CI (low and up)	Mean SD Median 95% CI (low and up)	Mean SD Median 95% CI (low and up)	Mean SD Median 95% CI (low and up)	Mean SD Median 95% CI (low and up)	Mean SD Median 95% CI (low and up)	Mean SD Median 95% CI (low and up)	Mean SD Median 95% CI (low and up)
Augmented soft tissue thickness (horizontal ridge width)	Width at 0 mm (µm)	2547.8 1714.7 2701.9 Low:1015.3 Up: 4080.3	2096.8 1602.0 2403.3 Low: 496.5 Up: 3697.1	168.9 239.7 0.0 Low: -45.4 Up: 383.1	1430.2 1369.4 833.9 Low: 206.3 Up: 2654.2	1862.6 1898.0 1450.6 Low: -33.4 Up: 3758.6	366.3 620.0 0.0 Low: -187.8 Up: 920.5	554.7 780.1 301.9 Low: -142.5 Up: 1251.8	231.3 318.5 0.0 Low: -53.3 Up: 515.9	71.8 190.0 0.0 Low: -98.0 Up: 241.6
	Width at 1.5 mm (µm)	1324.1 773.1 1233.7 Low: 633.1 Up: 2015.1	1371.8 683.2 1485.7 Low: 689.3 Up: 2054.2	248.2 350.3 70.0 Low: -64.9 Up: 561.3	816.8 665.5 655.9 Low: 222.1 Up: 1411.6	687.8 569.8 563.7 Low: 118.6 Up: 1256.9	345.8 433.2 130.9 Low: -41.4 Up: 733.0	635.9 227.7 644.7 Low: 432.5 Up: 839.4	679.3 596.7 674.9 Low: 146.0 Up: 1212.6	288.9 434.9 156.9 Low: -99.8 Up: 677.6
	Width at 3.5 mm (µm)	1352.3 751.2 1187.6 Low: 680.9 Up: 2023.7	1843.5 850.3 1542.7 Low: 994.1 Up: 2693.0	660.5 511.5 425.7 Low: 203.4 Up: 1117.7	804.4 569.2 739.8 Low: 295.7 Up: 1313.2	1160.4 489.2 1230.7 Low: 671.7 Up: 1649.2	435.2 317.5 305.0 Low: 151.5 Up: 718.9	701.9 229.6 617.3 Low: 496.7 Up: 907.1	810.8 285.2 841.9 Low: 555.8 Up: 1065.7	348.2 323.8 274.6 Low: 58.8 Up: 637.5
	Width at 5.5 mm (µm)	852.1 500.9 818.2 Low: 404.4 Up: 1299.8	1433.8 1042.6 1255.3 Low: 392.3 Up: 2475.3	524.0 413.1 377.8 Low: 154.8 Up: 893.3	594.2 595.1 420.0 Low: 62.3 Up: 1126.0	1208.6 426.7 1154.4 Low: 782.3 Up: 1634.9	641.2 382.3 585.6 Low: 299.6 Up: 982.8	647.4 299.5 671.1 Low: 379.7 Up: 915.0	466.4 196.4 472.1 Low: 290.9 Up: 642.0	324.4 83.8 309.2 Low: 249.5 Up: 399.2
	Width at 7.5 mm (µm)	494.7 330.0 541.8 Low: 199.8 Up: 789.6	897.1 869.6 528.6 Low: 28.4 Up: 1765.8	471.0 503.7 363.7 Low: 20.8 Up: 921.2	419.0 397.6 232.3 Low: 63.6 Up: 774.4	817.8 655.6 718.7 Low: 162.9 Up: 1472.7	374.7 240.9 377.7 Low: 159.4 Up: 590.1	314.3 243.9 287.1 Low: 96.3 Up:532.3	281.0 342.3 202.5 Low: -25.0 Up: 587.0	322.9 362.9 181.9 Low: -1.4 Up: 647.3

Table 2

Inter-group comparison ANOVA, Tukey, Games-Howell

		Augmented soft tissue thickness 0 mm µm	Augmented soft tissue thickness 1.5 mm µm	Augmented soft tissue thickness 3.5 mm µm	Augmented soft tissue thickness 5.5 mm µm	Augmented soft tissue thickness 7.5 mm µm
4 weeks	SCTG vs Control	p = 0.019*	p = 0.012*	p = 0.191	p =.655	p = 0.997
	SCTG vs VCMX	p = 0.877	p = 0.990	p = 0.443	p =.305	p = 0.459
	Control vs VCMX	p = 0.058	p = 0.011*	p = 0.019*	p = 0.069	p = 0.419
8 weeks	SCTG vs Control	p = 0.198	p = 0.287	p = 0.328	p = 0.982	p = 0.982
	SCTG vs VCMX	p = 0.889	p = 0.911	p = 0.377	p = 0.077	p = 0.276
	Control vs VCMX	p = 0.222	p = 0.530	p = 0.030*	p = 0.108	p = 0.209
24 weeks	SCTG vs Control	p = 0.190	p = 0.332	p = 0.071	p = 0.025*	p = 0.999
	SCTG vs VCMX	p = 0.459	p = 0.982	p = 0.754	p = 0.270	p = 0.979
	Control vs VCMX	p = 0.823	p = 0.253	p = 0.015*	p = 0.437	p = 0.968

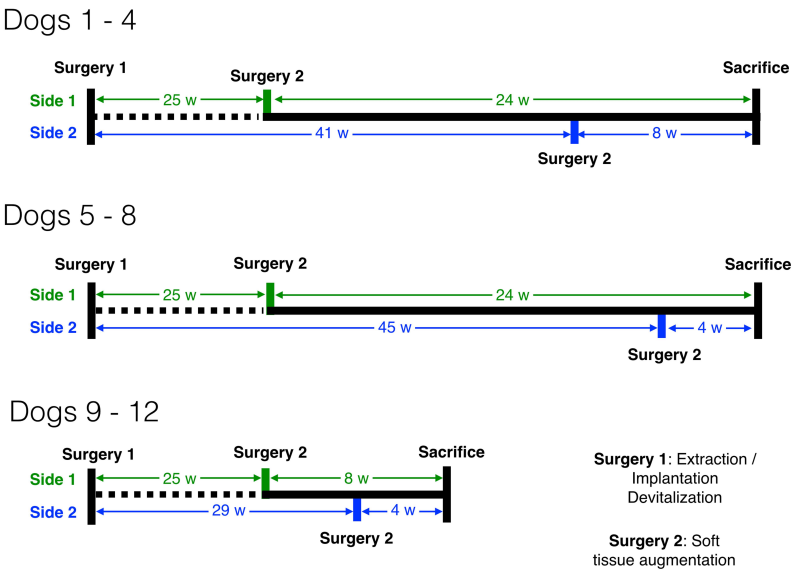

Figure 1

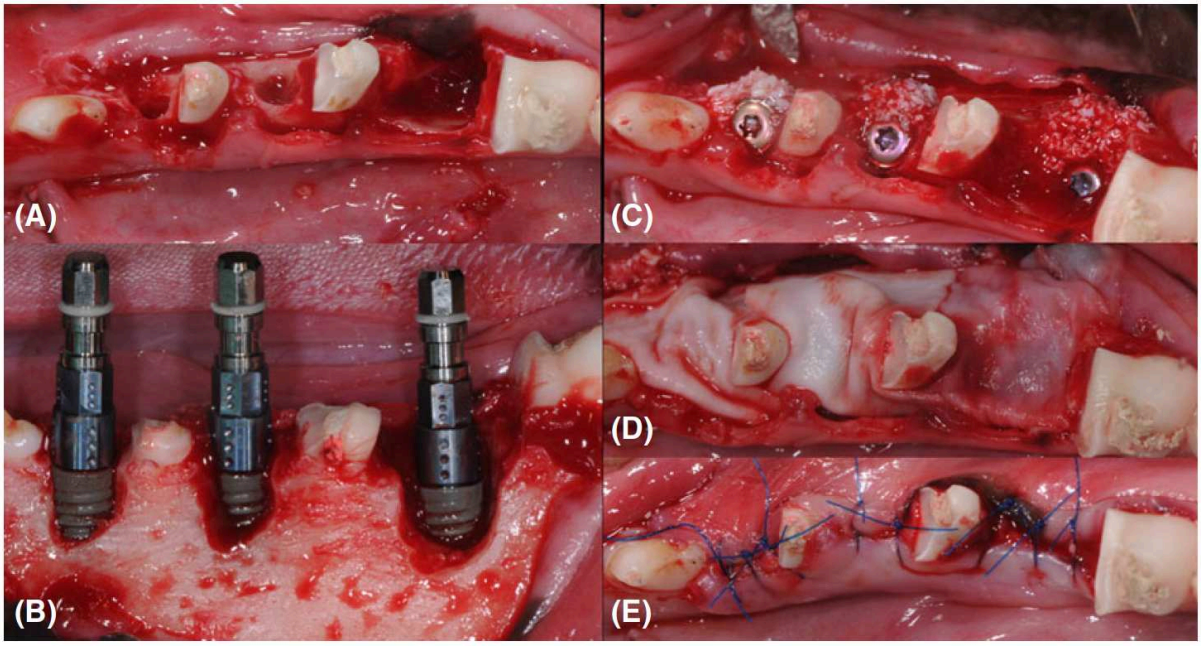

Figure 2

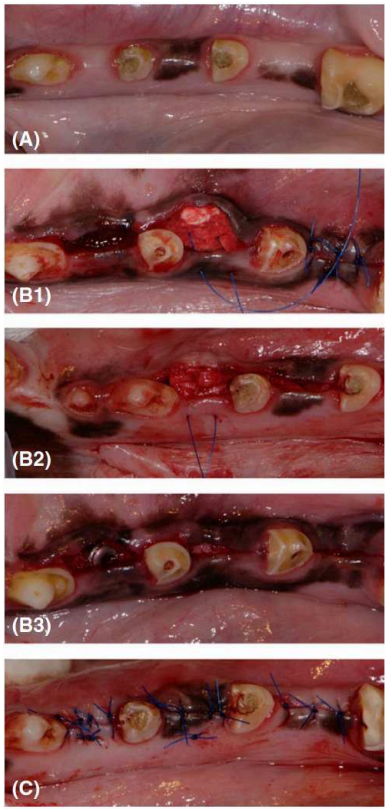

Figure 3

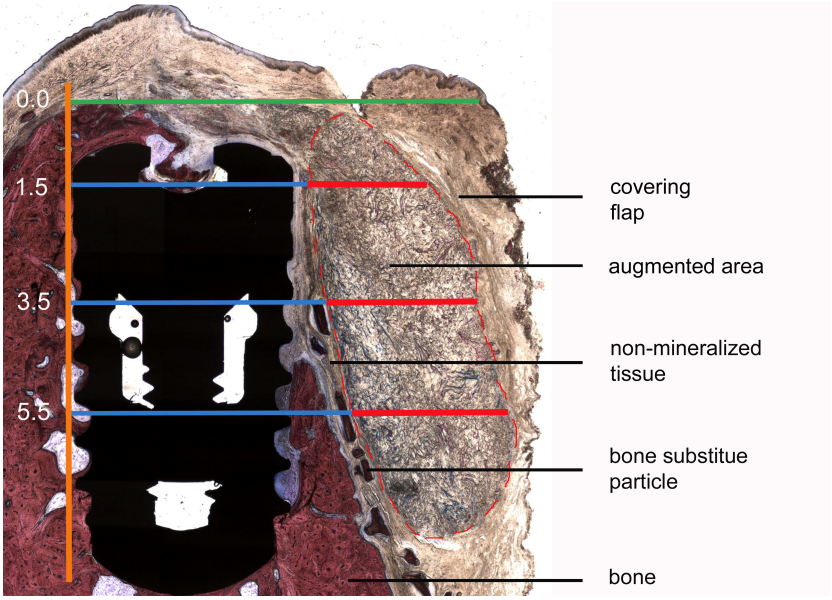

Figure 4

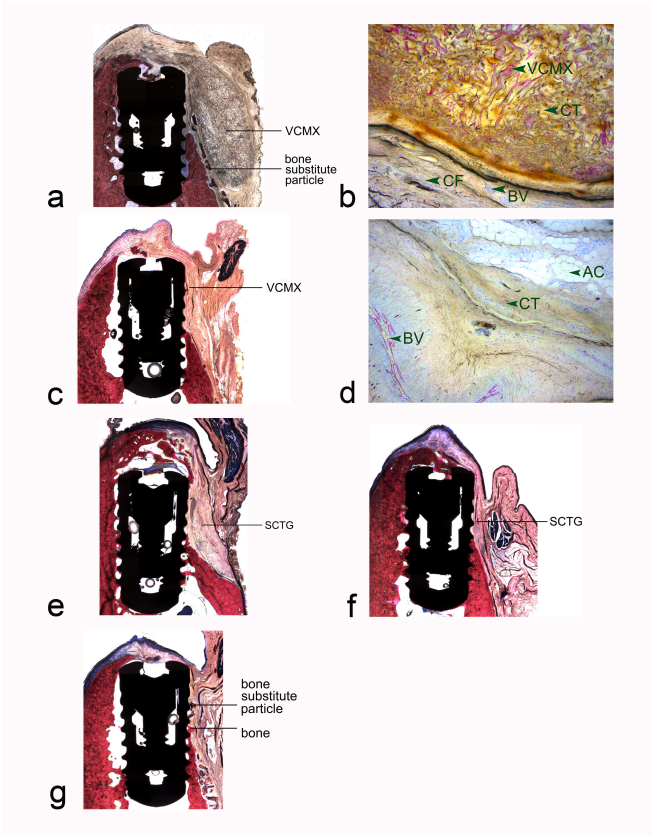
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Figure 5